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Seasonal changes in the concentration of some trace elements in macrophyte shoots

Sezonske spremembe v koncentracijah nekaterih elementov v sledovih v poganjkih makrofitov

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Key words: trace elements, sediment, Velenjsko jezero, *Myriophyllum*, *Najas*, *Potamogeton*, *Nuphar*

Abstract: Seasonal changes in the concentrations of trace elements (Ni, Cu, Pb, Zn, Cr and As) were analysed in shoots of macrophytes *Myriophyllum spicatum*, *Najas marina*, *Potamogeton crispus*, *Potamogeton lucens*, *Potamogeton nodosus*, *Potamogeton pectinatus* and *Nuphar lutea* taken from lake Velenjsko jezero (Slovenia), an artificial lake resulting from mining activity. Lake lies in the vicinity of the Šoštanj Thermal Power Plant, from which fly ash slurry was transported by pipeline and emptied into Velenjsko jezero until 1983. The degree of concentration of elements in plant's shoots varied according to the species of plant and the time of the season. The average concentrations of non-essential elements (Ni, Cr, Pb and As) in stems and leaves were the highest in *Myriophyllum spicatum* and the lowest in *Nuphar lutea*. Concentration of two essential elements Cu and Zn were the highest in the shoots of *Potamogeton lucens* and *Potamogeton crispus* respectively. Element concentration showed seasonal variation. For essential elements Zn and Cu there was a clear concentration decline from May to September in almost all macrophyte shoots, while non-essential elements remained at the same level or decreased through the season.

Izvleček: V pričujoči raziskavi smo spremljali sezonske spremembe v koncentraciji elementov v sledovih (Ni, Cu, Pb, Zn, Cr in As) v nadzemnih poganjkih makrofitov *Myriophyllum spicatum*, *Najas marina*, *Potamogeton crispus*, *Potamogeton lucens*, *Potamogeton nodosus*, *Potamogeton pectinatus* in *Nuphar lutea* iz umetnega Velenjskega jezera (Slovenija). Jezero leži v neposredni bližini Termoelektrarne Šoštanj. Do leta 1983 je jezero služilo kot odlagališče za pepel, ki je nastajal ob kurjenju premoga v termoelektrarni. Rezultati so pokazali, da so koncentracije elementov v rastlinah odvisne od vrste makrofitov in od časa v sezoni. Povprečne koncentracije ne-esencialnih elementov (Ni, Cr, Pb in As) so bile najvišje v nadzemnih delih vrste *Myriophyllum spicatum*, medtem ko so bile njihove najnižje koncentracije izmerjene v listih in steblih vrste *Nuphar lutea*. Najvišje povprečne koncentracije Cu so vsebovala nadzemna tkiva vrste *Potamogeton lucens*, medtem ko so bile najvišje koncentracije Zn izmerjene v tkivih vrste P*otamogeton crispus*. Sezonska nihanja v vsebnosti elementov so se razlikovala med vrstami makrofitov. Na splošno pa se je pri večini vrst pokazala težnja upadanja koncentracije esencialnih elementov, Zn in Cu, od maja do septembra, medtem ko je koncentracija ne-esencialnih elementov ostajala skozi celo sezono na isti ravni ali pa so koncentracije proti koncu sezone upadle.

Ključne besede: elementi v sledovih, sediment, Velenjsko jezero, *Myriophyllum*, *Najas*, *Potamogeton*, *Nuphar*

Abbreviations: Pot pec – *Potamogeton pectinatus*, Pot cri – *Potamogeton crispus*, Pot luc – *Potamogeton lucens*, Pot nod – *Potamogeton nodosus,* Myr spi – *Myriophyllum spicatum*, Naj mar – *Najas marina*, Nup lut – *Nuphar lutea*

Introduction

In aquatic system, where the input of pollutants is discontinuous and they are thus quickly diluted, analysis of plant tissues provides information about the quality of the system (Baldantoni et al. 2005). Aquatic plants can take up large quantities of trace elements (Jackson 1998, Hotzhina et al. 2001) from their environment, releasing them when they decay. While emergent plant species appear to enhance metal stabilization in the soil/sediment, submersed plants only slightly increase the retention of trace elements, by shoot accumulation (Fritioff et al. 2005). Abiotic factors such as pH, nutrient concentration in sediment and water, redox potential, water hardness, light, microbial activity and physical factors are very important in metal distribution in water and sediment and hence their availability to aquatic macrophytes (Guilizzoni 1991). Even if shoots and/or leaves of submersed plants accumulate lower concentrations of trace elements than roots (Mazej and Germ 2009), they may still provide reliable information, chiefly on water quality, over short periods equivalent to leaf age (Baldantoni et al. 2005). Further, the amounts of toxicants that reached the aboveground plant part are very important, because they can enter the food chains, presenting a potential danger for human and animal health. The concentration of trace elements in different parts of four macrophyte species (*Najas marina* L., *Potamogeton lucens* L., *Nuphar lutea* (L.) Sibth et Sm and *Potamogeton nodosus* Poir) from Velenjsko jezero has been already studied (Mazej and Germ 2009), however without seasonal dynamics. Metal accumulation may also be season dependent and knowledge about seasonal dynamics of element's concentrations in plant's shoots is useful in choosing appropriate plants and time for macrophyte phytoremediation systems. Reimer and Duthie (1993) reported that no significant trends were detected throughout the growing season in macrophyte concentrations of trace elements, but there have been reports of metal contents being highest during autumn and relatively low levels during spring (Brekken and Steinnes 2004).

The aim of this paper was to depict the seasonal dynamic in trace element content in above-ground tissues of *Najas marina*, *Myriophyllum spicatum* L., *Potamogeton crispus* L., *Potamogeton lucens*, *Potamogeton pectinatus* L., *Nuphar lutea* and *Potamogeton nodosus* from lake Velenjsko jezero.

Materials and Methods

Study area

Lake Velenjsko jezero is located in central Slovenia, in the Šalek Valley. It is situated at an altitude of 366 m, with a surface area of 135,000 m^2 and a maximal depth of 54 m. It is an artificial lake resulting from mining activity. As a result of subsidence, whole settlements, meadows and fields were submerged and flooded. Until 1983, fly ash slurry from the Šoštanj Thermal Power Plant was transported by pipeline and emptied into Velenjsko jezero. The large amount of ash (about 15 million tons) at the bottom of the lake is thus a source of trace elements (Ni, Cu, Pb, Zn, Cr in As) (Mazej et al. 2010), which can be accumulated through of their trophic chain.

Water, sediment and plant sampling

Seven native aquatic vascular plant species from Lake Velenjsko jezero were chosen: five entirely submersed (*Myriophyllum spicatum*, *Potamogetom crispus*, *Potamogeton pectinatus*, *Potamogeton lucens* and *Najas marina*) and two submersed with floating leaves (*Potamogeton nodosus* and *Nuphar lutea*). *Najas marina* is an annual and the dominant species of the littoral zone; other species are perennials and appear in the lake to a lesser extent.

Samples were taken monthly from May to September 2006. The three sampling locations were chosen, where macrophyte richness was greatest (1- 46°22'47.32''N, 15°05'46.90''E, 2 – 46°22'53.89''N, 15°05'26.85''E, 3 – 46°22'11.55''N, 15°05'07.80''E). The upper parts of the plants (leaves and stems) were collected by sampling rake. Sediment and water samples were collected at the same sampling locations. Samples of water were collected by hand from a boat by submerging precleaned polyethylene bottles approximately 50 cm beneath the water surface. Sediment was taken with a grab, at a depth from 0 to 10 cm.

Laboratory procedures and analyses

The collected lake water was acidified by 70 % super pure nitric acid (0.5 mL/100 mL sample) and used for heavy metal analyses. Sediment samples were dried, ground, and mixed to get an average sample.

Plant material, leaves and stems, were washed thoroughly with tap water to remove sediment and periphyton and rinsed with deionised water. Water was spilt with the paper from the plants, which were weighed and then dried to constant weight in an oven at 80 °C. This temperature was used because below this temperature all the moisture may not be removed and at higher temperatures thermal decomposition can reduce the dry weight (Peng et al. 2008). The dry samples were ground into a fine powder by milling (Büchi-Mixer B-400) and sieved at < 1 mm. A wet digestion procedure was used, involving concentrated nitric acid.

The contents of trace elements in lake water, sediment and plants were determined following the Hewlett Packard ICP-MS Application Note: 228–312, 228–314, 228–343 and a modified standard method: ISO 17294-2:2003 on Inductively Coupled Plasma-Mass atomic emission Spectrometry (ICP-MS 7500c-Agilent). The accuracy of chemical analyses was checked for plants using a standard reference plant material (IAEA-336, Trace and minor elements in Lichen). Recovery rates ranged from 95 %–100 % for all investigated elements. Analytical tolerance of method was 20 %.

Statistical analysis

Experimental data were analysed using the statistical software package Statistica for Windows 7.1 (StatSoft, 2006). For testing the differences in concentration of trace elements in the shoots of different macrophyte species and seasonal differences in the contents of trace elements in shoots, Mann-Whitney U test was used.

Results

Average concentrations of elements in water and sediment are presented in Tab. 1. Their mean concentrations in sediment decreased according to sequence Zn > Cr > Ni > Pb > Cu = As. Concentrations of trace elements in water were mainly below detection values, except for Cu, Pb and Ni, which were present in small, detectable concentrations. Plant shoots contained much smaller amounts of trace elements than sediment (Tab. 1, Figs. 1–2). Trace elements showed different degrees of accumulation in different species (Figs. 1–2, Tab. 2). In terms of annual average, *Potamogeton lucens* and *Potamogeton crispus* contained significantly more Cu and Zn in their above-ground tissues than other macrophyte species, but the differences between species were greater in the accumulation of other trace elements. *Myriophyllum spicatum* appeared to be the strongest accumulator of Pb, As, Ni and Cr. The accumulation of Cu was highest in *Potamogeton lucens* throughout the season. Ni was also relatively highly accumulated in *Potamogeton lucens* and *Potamogeton nodosus* and Pb in *Potamogeton crispus*. Pb was only accumulated to a small extent in *Potamogeton nodosus* and *Nuphar lutea*. *Nuphar lutea* was also the poorest accumulator of Ni and Cr.

Table 1: The average concentration of trace elements in sediment and in water sampled monthly in May, June, July and September 2006.

Tabela 1: Povprečne koncentracije elementov v sledovih v sedimentu in vodi, vzorčenih enkrat mesečno v mesecih maj, junij, julij in september.

	Sediment (mg/kg dw) n=12	Water (μg/L) n=12
As	10.6±3.08	<1.0
Cr	39.0±5.21	<5.0
Zn	90.9±12.2	<2.0
Ni	20.6±2.68	2.0±0.1
Pb	16.1±1.63	<0.5
Cu	12.1±2.79	1.1±0.05

dw – dry weight

The concentrations of trace elements in plant shoots showed different seasonal variations (Figs. 1–2). Cu and Zn concentrations decreased from May to September in all species studied, except in *Najas marina*, which was present in the lake only from July to September. In other species Pb, As, Ni and Cr showed different patterns. The variations were high, even within plants of the same

Table 2: Mann-Whitney U test of differences between the average concentrations of trace elements in the above-ground tissues of macrophytes. Different superscript letters within each column indicate significant differences at $p < 0.05$. Shaded cells mark species that contain the significant highest concentration of the metal.

Tabela 2: Razlike med povprečnimi koncentracijami elementov v nadzemnih tkivih makrofitov (Mann-Whitney U test). Različne nadpisane črke kažejo na značilne razlike pri $p < 0.05$. Senčene celice označujejo vrste makrofitov, ki so vsebovale značilno najvišje koncentracije elementov v svojih nadzemnih tkivih.

Pb	As	Ni	Cr	Cu	Zn
Myr spi^{a}	Myr spi^{a}	Myr spi^{a}	Myr spi^{a}	Pot luc^{a}	Pot cri^{a}
Pot cri^{a}	Naj mar^{a}	Pot nod^{a}	Naj mar^{ab}	Pot cri^{b}	Myr spi^{b}
Naj mar^{b}	Pot cri^{bc}	Pot pect^{a}	Pot cri^{b}	Pot nod^{b}	Pot pect^{bc}
Pot luc^{b}	Nup lut^{bc}	Naj mar^{ab}	Pot nod^{b}	Pot pect^{b}	Pot luc^{bc}
Pot pec^{b}	Pot nod^{bc}	Pot luc^{b}	Pot pect^{a}	Nup lut^{b}	Nup lut^{bc}
Nup lut^{b}	Pot pect^{bc}	Pot cri^{b}	Pot luc^{bc}	Naj mar^{c}	Pot nod^{bc}
Pot nod^{b}	Pot luc^{bc}	Nup lut^{c}	Nup lut^{c}	Myr spi^{c}	Naj mar^{c}

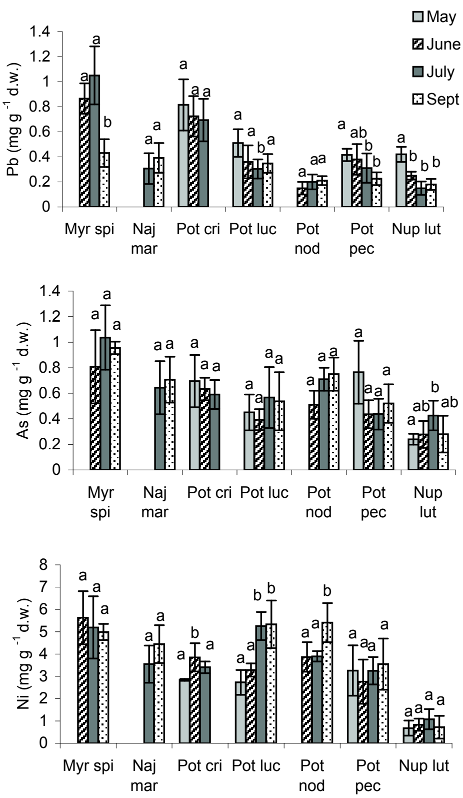

Figure 1: Average concentrations ($mg\ kg^{-1}$ dry weight) of Pb, As and Ni in plant's shoots of macrophyte species together with standard deviation (SD) bars. Different letters in each group indicate significant differences at $p<0.05$.

Slika 1: Povprečne koncentracije ($mg\ kg^{-1}$ suhe teže) Pb, As and Ni v nadzemnih tkivih sedmih vrst makrofitov skupaj s standardno deviacijo. Različne nadpisane črke kažejo na značilne razlike pri $p < 0.05$.

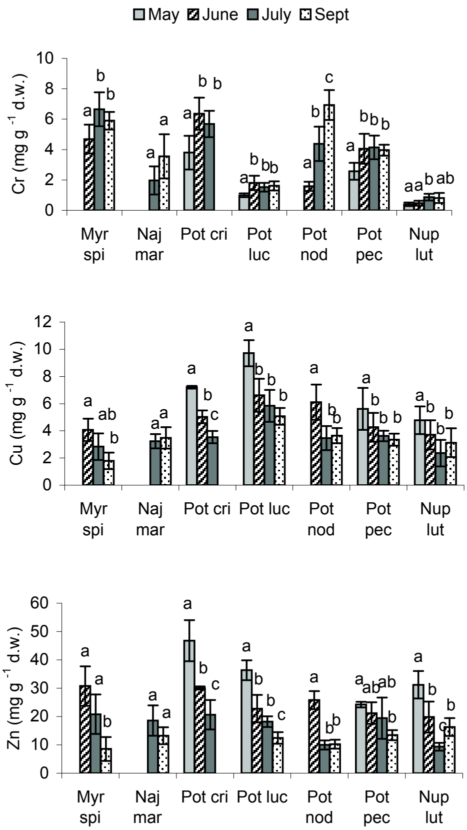

Figure 2: Average concentrations (mg kg^{-1} dry weight) of Cr, Cu and Zn in plant's shoots of macrophyte species together with standard deviation (SD) bars. Different letters in each group indicate significant differences at $p<0.05$.

Slika 2: Povprečne koncentracije (mg kg^{-1} suhe teže) Cr, Cu and Zn v nadzemnih tkivih sedmih vrst makrofitov skupaj s standardno deviacijo. Različne nadpisane črke kažejo na značilne razlike pri $p < 0.05$.

species. The concentration of Pb decreased from May to September in the shoots of *Myriophyllum spicatum*, *Potamogeton pectinatus* and *Nuphar lutea*, whereas there were no seasonal differences in the other species. Seasonal differences were also not observed in the case of As; only in the shoots of *Nuphar lutea* its concentration increased in July. The tissue concentration of Cr increased in midsummer. An increase in Ni content was observed at the end of the summer in the shoots of *Potamogeton crispus* and *Potamogeton nodosus*. In other species the concentrations were stable throughout the season.

Discussion

Previous results showed that Only the concentration of As in sediment from Velenjsko jezero was above European background values, while concentrations of Cr, Pb, Zn, Cu and Ni in sediments were within the ranges typical of European background values from unpolluted sites (Mazej and Germ 2009). Total concentrations of soluble trace elements in water were very small, probably because a sedimentary process takes place and the less soluble forms are accumulated in the suspended or sedimented phase (Duman et al. 2006). This indicates that the quantity of trace elements in above-ground parts of plants is predominantly the result of their translocation from roots. Uptake by leaves becomes more important when the metal concentrations in the surrounding environment are high (Guilizzoni 1991), which was not the case in lake Velenjsko jezero. Zn and Cu, two essential elements for plants, were translocated from the sediment to the shoots to a higher degree than toxic trace elements (As, Pb, Ni and Cr) (Mazej and Germ 2009). The concentration of Pb was the lowest of all the trace elements in above-ground tissues of the species studied (Fig. 1). This is probably due to its strong binding to the organic matter in sediment and other components (Adriano 1986) and its binding to the roots (Sinicrope et al. 1992) so that it remains at the uptake site.

This investigation as well as previous (Mazej and Germ 2009) showed that submersed species *Myriophyllum spicatum*, with dissected leaves, was among seven investigated macrophyte species the strongest accumulator of toxic trace elements (Pb, As, Ni, Cr) in its shoots, and *Nuphar lutea* the poorest. This excessive metal uptake by *Myriophyllum spicatum*, followed closely by *Najas marina* and *Potamogeton crispus*, may be a function of the growth form of these truly submerged species, with no floating or emergent components (Cardwell et al., 2002). Entirely submersed species have been shown also to accumulate relatively large amounts of trace elements (e.g. Jackson and

Kalff 1993, Fritioff and Greger 2003, Fritioff et al. 2005, Mazej and Germ 2009).

The concentration of trace elements in plants undergoes substantial changes according to the season (Guilizzoni 1991). Some publications have reported the highest metal contents during autumn and relatively low levels during the spring (Brekken and Steinnes 2004, Kim and Fergusson 1994), whereas others have indicated the highest foliar levels during the spring and lowest during the winter (Martin and Couphtrey 1982, Wilkins 1978). Gommes and Muntau (1981) stated a rule that concentration in the plant population is highest in the cold season, decreasing rapidly during the period of maximal growth. Only spring and summer were included in our research and seasonal pattern of all elements studied are similar in almost all above-ground tissues of plant species. Concentrations of Zn and Cu decreased from May to September, while concentrations of non-essential elements remained at the same level or decreased. Duman et al. (2007) observed clear concentration decline of trace elements (Pb, Cr, Cu, Mn, Ni, Zn, Cd) from the spring to the summer in the shoots of aquatic macrophytes *Phragmites australis* (Cav.) Trin. Ex Steudel and *Schoenoplectus lacustris* (L.) Pall. Duman et al. (2006) reported that in shoots of *Potamogeton lucens*, Cr, Ni and Cd concentrations increased during summer while Pb, Cu and Zn concentrations fell during the summer in comparison to spring but Pb concentrations built up again during senescence (Duman et al. 2006). The same pattern was observed also for the same species from Velenjsko jezero. The essential elements, Zn and Cu, showed seasonal pattern similar to that of the nutrients nitrogen and phosphorus (Furtado 1998, Garbey et al. 2004, Mazej and Germ 2005). Spring growth is characterized by a rapid uptake of nutrients and trace elements (Duman et al. 2006). Later in the season, during the active period of growth, dilution of nutrients occurs (Nan et al. 2002, Villares et al. 2002) and at the end of season, in September, the nutrient concentrations in plant parts decline, because of a large decrease in metabolic rates.

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References

Adriano, D.C., 1986. Trace elements in Terrestrial Environment. Springer-Verlag, New York.

Baldantoni D., Maisto G., Bartoli G. & A. Alfani 2005: Analyses of three native aquatic plant species to assess spatial gradient of lake trace element contamination. Aquatic Botany, 83, 48–60.

Brekken, A., Steinnes, E., 2004. Seasonal concentration of cadmium and zinc in native pasture plants: consequence for grazing animals. Science of the Total Environment, 326, 181–195.

Cardwell, A.J., Hawker, D.W., Greenway, M., 2002: Heavy metal accumulation in aquatic macrophytes from southeast Queensland, Australia. Chemosphere, 48, 653–663.

Duman, F., Obali, O., Demirezen, D., 2006. Seasonal changes of metal accumulation and distribution in shining pondweed (*Potamogeton lucens*). Chemosphere, 65, 2145–2151.

Duman F., Cicek, M., Sezen, G., 2007. Seasonal changes of metal accumulation and distribution in common club rush (*Schoenoplectus lacustris*) and common reed (*Phragmites australis*). Ecotoxicology, 16, 457–463.

Fritioff, Å., Greger, M., 2003. Aquatic and terrestrial plant species with potential to remove trace elements from stormwater. International Journal of Phytoremediation, 5, 211–224.

Fritioff, Å., Kautsky, L., Greger, M., 2005. Influence of temperature and salinity on heavy metal uptake by submersed plant. Environmental Pollution, 133, 265–274.

Furtado, A.L.S., 1998. Ash free dry weight, organic carbon, nitrogen and phosphorus content of *Typha domingensis* Pers. (Thyphaceae) and aquatic macrophyte. Verhandlungen – Internationale Vereinigung für Limnology, 26, 1842–1845.

Garbey, C., Murphy, K.J., Thiébaut, G. Muller, S., 2004. Variation in P-content in aquatic plant tissues offers an efficient tool for determining plant growth strategies along a resource gradient. Freshwater Biology, 49, 346–356.

Gommes, R., Muntau, H., 1981. Variation saisonnière de la composition chimique des limnophytes du Lago Maggiore. Memorie dell'Istituto Italiano di Idrobiologia, 38, 309–330.

Guilizzoni, P., 1991. The role of trace elements and toxic materials in the physiological ecology of submersed macrophytes. Aqutic Botany, 41, 87–109.

Hotzina, E.I., Khramov, A.A., Gerasimov, P.A., Kumarkov, A.A., 2001. Uptake of trace elements, arsenic and antimony by aquatic plants in the vicinity of ore mining and processing industries. Journal of Geochemical Exploration, 74, 153–162.

Jackson, L.J., Kalff, J., 1993. Patterns in heavy metal content of submerged aquatic macrophytes: the role of plant growth form. Freshwater Biology, 29, 351–359.

Jackson, L.J., 1998. Paradigms of metal accumulation in rooted aquatic vascular plants. Science of the Total Environment, 219 (2–3), 223–231.

Kim, N.D., Fergusson, J.E., 1994. Seasonal variations in the concentration of cadmium, copper, lead and zinc in leaves of the horse chestnut (*Aesculus hippocastanum* L.). Environmental Pollution, 86, 89–97.

Mazej, Z., Germ, M., 2008. Seasonal changes in the contents of nutrients in five macrophyte species from the lake Velenjsko jezero (Slovenia) = Sezonske spremembe vsebnosti hranil v petih vrstah makrofitov iz Velenjskega jezera (Slovenija). Acta Biologica Slovenica, 51(1), 3–11.

Mazej, Z., Germ, M., 2009. Trace element accumulation and distribution in four aquatic macrophytes. Chemosphere, 74, 642–647.

Mazej, Z., Al Sayegh Petkovšek, S., Pokorny, B., 2010. Heavy metal concentrations in food chain of lake Velenjsko jezero, Slovenia: an artificial lake from mining. Archives of Environmental Contamination and Toxicology, 58, 998–1007.

Martin, M., Couphtrey, P. 1982: Biological monitoring of heavy metal pollution. Applied Sciences Publications, London, New York, 475 pp.

Nan, Z., Li, J., Zhang, J., Cheng, G., 2002. Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. Science of the Total Environment, 285, 187–195.

Peng, K., Luo, C., Lou, L., Li, X., Shen, Z., 2008. Bioaccumulation of trace elements by the aquatic plants *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. and their potential use for contamination indicators and wastewater treatment. Science of the Total Environment, 392, 22–29.

Reimer, P., Duthie, H.C. 1993. Concentration of Zn and chromium in aquatic macrophytes from the Sudbury and Muskoka regions of Ontario, Canada. Environmental Pollution, 79, 261–265.

Sinicrope, T.L., Langis, R., Gersberg, R.M., Busanardo, M.J., Zedler J.B., 1992. Metal removal by wetland mesocosms subjected to different hydroperiods. Ecological Engineering, 1, 309–322.

Villares, R., Puente, X., Carballeira, A., 2002. Seasonal variation and background levels of trace elements in two green seaweeds. Environmental Pollution, 119, 79–90.

Wilkins, D.A., 1978. The measurement of tolerance to edaphic factors by means of root growth. New Phytology, 80, 623–633.